OL. VIII, No. 9 NOVEMBER, 1947

BORDEN'S REVIEW *of* NUTRITION RESEARCH

THE NUTRITIONAL ROLE OF LIPOTROPIC FACTORS

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

THE NUTRITIONAL ROLE OF LIPOTROPIC FACTORS

By RUTH WOODS

The importance of the liver in the body economy has been recognized since the beginnings of human physiology. The role of the liver in fat metabolism constitutes one of its many vital functions. Recent researches in physiology, nutrition and medicine have contributed significantly to an understanding of the complexities of liver function in fat metabolism. One of the most interesting aspects of these researches concerns the concept that certain components of the diet, described collectively as "lipotropic" (literally, fat turning) factors play an important part in controlling the metabolism and deposition of fat in the liver. This concept has assumed practical significance in providing a rational basis for nutrition therapy in human liver disorders associated with fat metabolism.

Historical Background:

The story of the lipotropic factors in food has its origin in the discovery of insulin by Banting and Best at the University of Toronto in Canada. In the course of studies with insulin, the anti-diabetic hormone secreted by normal pancreas, experiments were conducted on dogs in whom diabetes was induced by removal of the pancreas. Prolonged experiments of this type conducted by various other investigators soon brought to light the fact that depancreatized dogs adequately supplied with insulin, although free from diabetes, developed another disorder characterized by the appearance of enlarged yellow, fatty livers (1, 2). On the theory that removal of the pancreas caused a deficiency of digestive enzymes and therefore interfered with the absorption of foodstuffs, raw pancreas were fed to these animals and were found to prevent the abnormal accumulation of fat in the liver.

About six years later, Hershey (3) suggested the use of the phospholipid, lecithin as a substitute for raw pancreas and, together with Soskin (4) reported its effectiveness in treating the disorder. Hershey's use of lecithin was predicated on the theory that the development of fatty livers reflected a derangement of fat metabolism in the liver rather than a disturbance in digestion due to lack of enzymes. In view of the existing theory that phospholipids were involved in fat transport from the liver, Hershey decided to feed a phospholipid—lecithin—to the depancreatized animals in order to provide an essential factor for fat transport and metabolism.

On the basis of this work, Best, Hershey and Huntsman (5) proceeded to investigate more thoroughly the subject of factors which influence the deposition of fat in the liver. Because work with depancreatized dogs is

extremely time-consuming and expensive, the experimental studies were transferred to rats. It was found that a similar type of fatty liver could be induced in rats by feeding a diet exceedingly rich in fat, thereby obviating the necessity for removal of the pancreas. In 1932, Best and coworkers reported the effectiveness of both crude and highly purified lecithin in treating fatty livers in rats on high fat diets. In subsequent studies on the components of lecithin, they found the active constituent to be choline* (6, 7). If given in sufficient amounts, choline was found to prevent the fatty livers of depancreatized dogs as well, and to cure the condition after it had developed (8).

The term *"lipotropic"* was introduced to describe the action of choline in the prevention and cure of fatty livers (9) and has since come to refer to other substances also which prevent or remove an accumulation of excess fat in the liver.

In the light of subsequent studies and more recent theories, it may be that there was more truth than realized in the original hypothesis that the lack of digestive enzymes was the important factor in the development of fatty livers in depancreatized dogs. Studies in the last few years have demonstrated the existence in both raw pancreas and pancreatic juice of a lipotropic factor, *other than choline*, which strongly resembles or may even be identical with one or more proteolytic enzymes. It has been postulated, therefore, that the anti-fatty liver factor of pancreas may exert its effect by permitting the enzymatic liberation of lipotropic materials present in dietary proteins. (In particular, the liberation of the lipotropic amino acid, methionine, from casein and other proteins.)† This phase of the subject will be reviewed in detail further on in this paper.

Types of Fatty Livers:

Before going on to discuss the various lipotropic factors and the theories regarding their behavior, it is advisable to point out that there are several distinct types of fatty livers resulting from a number of diverse causes. Briefly enumerated, these are:

1. Fatty livers induced by dietary means such as:
   a. Ingestion of a low protein, low choline diet.
   b. Ingestion of extremely high fat diet.
   c. Ingestion of excessive amounts of cholesterol.
   d. Ingestion of certain alcohol extracts of raw meat and of raw dried liver.
   e. Ingestion of certain of the B complex vitamins (probably indirect effect).
2. Fatty livers induced in dogs by removal of the pancreas.
3. Fatty livers resulting from starvation.

---

*The chemically related compound, betaine, was found to be active also but to a lesser degree.

†Since the function of methionine is to supply methyl groups for the synthesis of choline in the body, it is apparent that both hypotheses were reasonable. On the one hand, digestive enzymes released lipotropic material and on the other hand, lipotropic material (in lecithin) was supplied directly.

4. Fatty livers resulting from ingestion of certain poisons (such as carbon tetrachloride).
5. Fatty livers induced by hormones (anterior pituitary extract).

A fatty liver is characterized by the infiltration and deposition of excessive quantities of fat. The fat deposit is divided largely between neutral or glyceride fat and bound sterol fat in the form of cholesterol esters. Fatty livers differ in type essentially in the ratio of glyceride to sterol fat. Thus in the fatty livers induced by high fat feeding, low protein diets, choline deficiency, or by thiamine or biotin, the pathological picture consists of a predominance of glyceride fat and a relatively small amount of cholesterol esters. In the fatty livers induced by an intake of excessive amounts of cholesterol, cholesterol and sterol esters may predominate over glycerides although Best *et al* (9a, 9b) have found that, even with intakes of cholesterol up to 1.5 per cent of the diet, glyceride fats predominate over cholesterol in the resultant fatty liver.

There is considerable difference of opinion as to the types of fatty liver caused by certain of the above factors. It has been pointed out recently, for example, that the so-called "biotin fatty liver" and the fatty liver induced by high fat diets are not really distinct entities as previously thought, but are actually similar in nature and respond to treatment with the same type of lipotropic factors (10).

Types of Lipotropic Factors:

Following the discovery of the lipotropic activity of choline and related compounds, it was soon found that other types of compounds also possessed lipotropic activity. Prominent among these are certain of the *proteins,* notably casein; the amino acid, *methionine* which, to a large extent, determines the lipotropic behavior of the proteins; certain of the vitamins such as inositol and pyridoxine; and two separate unidentified fractions extracted from pancreas, Dragstedt's *lipocaic* (11) and the so-called *anti-fatty liver fraction* (AFL) of Entenman, Chaikoff and Montgomery (12, 13). The AFL factor has been reported to occur in pancreatic juice as well as in pancreatic tissue. Lipocaic is believed not to be present in pancreatic juice.

Lipotropic Action of Choline:

Choline is recognized as an effective lipotropic agent in the fatty liver of rats induced by feeding a high fat diet (5). In somewhat larger amounts it has been shown to be effective in preventing and curing the fatty livers encountered in depancreatized dogs maintained with insulin (8, 12). The so-called "biotin fatty liver" formerly thought to be resistant to choline therapy (13a) was recently shown to be similar in character to the "fat"

fatty liver and to respond to choline (10, 10a). McHenry and Gavin (14), also working on this problem at the University of Toronto, originally presented evidence indicating that inositol exerted a marked lipotropic effect on the "biotin fatty liver." This has been recently interpreted by Best *et al* (10) as a supplementary effect to that of the choline which was present in the basal diet used by Gavin and McHenry.

Best, Channon and Ridout (15, 16) found that choline prevented the deposition of neutral fat (glycerides) and to a lesser extent the cholesterol fat in the so-called "cholesterol fatty liver." In curative experiments choline accelerated the removal of cholesterol esters as well as glycerides from the liver.

In 1937, McHenry (17) reported the existence of an apparent relation between choline and thiamine in the production of fatty livers. He found that it was difficult to produce fatty livers in rats fed choline-free diets unless thiamine was supplied in the diet. When the diet contained more than 40 per cent of fat, there was a significant increase in liver fat in the absence of added thiamine, but at lower levels of fat intake, fatty livers did not occur—even in the absence of choline — *unless thiamine was available.* McHenry *et al* (18) believed that the effect of thiamine might be due to its possible influence in stimulating fat synthesis from dietary carbohydrate. Later work by Boxer and Stetten (18a), however, seems to indicate that the effect of thiamine is essentially an indirect one resulting from its action in stimulating appetite and thus increasing the need for lipotropic factors. These so-called "thiamine fatty livers" are identical in nature to those caused by simple high fat feeding and respond similarly to administration of choline.

The interrelations between choline and inositol, choline and thiamine, and the various other members of the vitamin B complex will be discussed more fully in another section of this Review.

Lipotropic Action of Proteins:

Three years after their discovery that choline was the active lipotropic component of lecithin, Best and Huntsman (19) made the interesting observation that the milk protein, casein, exerted a similar effect. This effect was noted during a study of the curative effect of choline on the fatty liver of rats in various states of nutrition. In one experiment in this series, such rats were placed on a diet containing only sugar (no source of choline added) and were found to develop an even higher fat level in the liver. The replacement of 20 per cent of the sugar by casein prevented this increase in liver fat.

This work was taken up and further investigated by Channon *et al*

in England. Six months after the initial report by Best and Huntsman, the British workers (20) published results of studies with rats fed a low choline, high fat (40 per cent) diet supplemented with increasing amounts of casein (from 5 to 50 per cent of the diet). They found that as the amount of casein in the diet increased, the liver fat decreased. The maximal lipotropic effect of casein in a 40 per cent fat diet was obtained when the protein comprised 30 per cent of the diet, according to subsequent reports by Best *et al* (21). The British workers (22) found that the lipotropic effect *(preventive)* of casein was exerted on the glyceride fraction of cholesterol fatty livers. The group in Toronto (23), in *curative* experiments on cholesterol fatty livers found that casein decreased the cholesterol fraction as well. (It may be of some significance that the British group had fed about ten times as much cholesterol [2 per cent of diet] as did the Toronto group.)

Effect of Various Amino Acids: The discovery of the lipotropic effect of casein naturally prompted an inquiry into which of its component amino acids, if any, was responsible for this effect. Both the Canadian and British groups (19, 20) had suggested that the lipotropic action of casein might be due to the metabolic conversion of certain of its amino acids to betaines, or perhaps even choline (20).

In a preliminary study, Beeston and Channon (24) were unable to observe a lipotropic effect with any of the following amino acids: Lysine, glutamic acid, aspartic acid, serine, glycine and phenylalanine. The ineffectiveness of a number of other amino acids was demonstrated in a later study. They did note the surprising fact, however, that the sulfur-containing amino acid, cystine, exerted a pronounced *anti-lipotropic* effect on a low casein diet. This effect was negated by raising the casein level to 30 per cent.

The following year a significant advance in the problem was made when Tucker and Eckstein (25) reported the lipotropic nature of the amino acid, methionine (another sulfur-containing amino acid), and confirmed the anti-lipotropic nature of cystine. They attributed the lipotropic activity of casein and other proteins to the content of methionine and postulated further that the methionine-cystine ratio of a protein or of the diet might be an important factor in determining the degree of lipotropic activity.

The Cystine-Methionine Relationship: There are two major theories, backed by a considerable mass of contradictory experimental evidence, as to the lipotropic effect of the proteins on the basis of their cystine and methionine contents. One theory suggests that since cystine and methionine have opposite effects a direct antagonism exists between the two amino acids (26), thereby mutually modifying their actions in the diet. On the

other hand there is considerable evidence that cystine raises the level of metabolism and stimulates growth, thereby creating an increased demand for methionine for growth purposes and consequently reducing the amount of methionine available for lipotropic purposes (26, 27, 28).

The conflicting results reported by various groups of investigators are due in large part to the marked divergence in experimental conditions. Different levels of casein were used by the individual groups; varying limitations on total food intake were set; varying proportions of cystine and methionine were provided. Inasmuch as all of these factors influence lipotropic activity, it is difficult to correlate results of experiments conducted under such highly unstandardized conditions.

An investigation of the lipotropic activity of various proteins (milk, casein and albumin, beef muscle, wheat edestin, blood fibrin, wheat gliadin, etc.) by Channon *et al* (29) demonstrated marked differences in activity. Tucker and Eckstein (30) subsequently pointed out that the lipotropic activity of these proteins paralleled in general their methionine content.

It is now generally believed that the lipotropic activity of the proteins may be largely explained on the basis of their methionine content. The lipotropic activity of methionine itself, however, is another problem the answer to which was provided by the very interesting studies of du Vigneaud and his collaborators.

Mechanism of Methionine Activity: The explanation of the lipotropic effect of methionine came unexpectedly from a field of investigation quite removed from the studies on fatty livers and choline. This story has been told in considerable detail in previous issues of this Review* and will, therefore, be only sketchily repeated here.

During the course of studies on the three sulfur-containing amino acids, cystine, homocystine and methionine, du Vigneaud *et al* (31, 32) discovered that the non-essential amino acid homocystine could replace the essential methionine as a growth factor for rats *only if small amounts of choline were present in the diet.* In an attempt to explain this phenomenon, the following facts were examined:

1. The only difference between methionine and homocystine is the presence of a methyl group in the former compound.
2. Choline is a compound containing methyl groups.
3. Triethylcholine (choline in which the methyl groups are replaced by ethyl groups) has lipotropic activity. It prevents the fatty livers which occur on a homocystine diet, but cannot replace choline as a growth supplement to homocystine in the diet of rats.

---

*cf. Borden's Review of Nutrition Research, October, 1942 and September, 1946—"Labile Methyl Groups of Protein and Choline," and "Labile Methyl Groups in Nutrition — New Findings," respectively.

Reasoning from these facts, du Vigneaud's group concluded that the effect of choline was not due to its lipotropic activity, but quite possibly to the fact that choline donated a methyl group* to homocystine, thereby converting it to methionine. The validity of this assumption was soon confirmed through the use of isotopically "tagged" compounds, thereby establishing the occurrence of a *reversible* transmethylation reaction between methionine and choline (33, 34).

Here then was the explanation of the lipotropic activity of methionine. Given choline and homocystine, the animal synthesized methionine and was able to grow; on the other hand, *given methionine, the animal was able to synthesize choline* and thus protect itself against the development of fatty liver.

In subsequent studies Chandler and du Vigneaud (35) showed that betaine permits the rat to utilize homocystine in place of methionine, but less effectively than does choline. The explanation offered by these workers is that choline is the compound which is actually used by the body for the methylation of homocystine. Betaine therefore acts indirectly by providing methyl groups for the eventual synthesis of choline which, in turn, provides methyl groups for the conversion of homocystine to methionine. This explanation was subsequently confirmed by Stetten (36) using isotopic nitrogen (N_{15}) as a tracer. He found that betaine containing N_{15} was not directly converted to choline, but rather was demethylated to form glycine rich in N_{15}. Some of the N_{15} of betaine found its way into ethanolamine tissue phospholipids and it is this compound, when methylated, which yields choline.

Lipotropic Action of Pancreas Factors:

During the early insulin studies on depancreatized dogs it was noted by various investigators that the feeding of raw pancreas had marked lipotropic value. The finding by Best and Huntsman (6) that not only choline, but betaine was lipotropically active, and later that the protein casein was active (19) indicated that several different types of compounds possessed lipotropic activity. The possibility existed, therefore, that the lipotropic activity of the pancreas might be due not only to its choline content, but to the presence of some other lipotropically active compound as well.

Lipocaic: The first indication that such a compound might be present in pancreas came in 1936 when Dragstedt, Van Prohaska and Harms (37, 38)

---

*Homocystine consists of two molecules of homocystine which latter amino acid is the non-methylated form of methionine. Accurately speaking, therefore, choline donates *two* methyl groups to homocystine.

postulated the existence of "lipocaic," an unidentified material extracted from pancreas. The amount of pancreatic material which Dragstedt *et al* found to be lipotropically active was much smaller than would be needed on the basis of its choline content. They interpreted this as conclusive evidence of the existence of a lipotropic factor other than choline. Dragstedt *et al* have suggested that lipocaic may be another hormone of the pancreas, like insulin, but this view has been refuted by other investigators (39, 40, 41, 42, 43).

Anti-Fatty Liver Factor: In similar studies on the lipotropic effect of pancreas, Kaplan and Chaikoff (44, 45, 46) made a number of interesting observations concerning the level of fat which accumulated in the blood as well as in the liver of depancreatized dogs. They postulated the existence of two factors in raw pancreas, one which controls blood lipid levels and another (probably choline) which controls liver fat deposition. These investigators further pointed out that the differences in cause and type of fatty livers in depancreatized dogs and in normal rats on a high fat diet should be taken into account in evaluating results and in applying the findings with one species to the other:

> "Although choline influences the fatty livers of rats fed a high-fat, low protein diet as well as those of depancreatized dogs maintained on a *low-fat, high protein diet,** the evidence available at present does not permit the conclusion that the mechanism of production and cure of these two types of fatty livers is the same. Hence conclusions derived from experiments on rats cannot be applied at present to depancreatized dogs."

In subsequent studies by Chaikoff's group, the lipotropic factor of the pancreas was shown to be a substance distinct from choline and also from lipocaic (13, 47). It was found to be present in pancreatic juice as well as in raw pancreas (47).

Because subsequent investigators in large part did not attempt to segregate results of experiments with rats and with depancreatized dogs as suggested by Kaplan and Chaikoff, a great deal of confusion has arisen with respect to the activity and function of the various lipotropic factors. Further, there has been little attempt to standardize experimental conditions among the several independently working laboratories even with the same experimental animal. Thus, for example, in studies with depancreatized dogs, some animals were maintained on high protein, low fat diets without vitamin supplements; others had high protein, adequate fat diets; still others had high protein, low fat diets generously supplemented with B vita-

---

**Editor's Note:* It is significant that in normal rats (i.e. having intact pancreas) a low-fat, high protein diet is *protective* against fatty livers. This interesting difference has aided in the recent elucidation of the nature of the anti-fatty liver factor (other than choline or lipocaic) in raw pancreas.

mins. Since thiamine, biotin and inositol are three vitamins which are believed to alter significantly the lipotropic effect of a diet, it can readily be seen how difficult it has been to correlate and interpret the results of the individual research groups.

Possible Lipogenic Factors:

Of considerable interest in the study of lipotropic factors is the postulated existence of two unidentified factors in food which may exert a positive causative effect on the formation of fatty livers in experimental animals. These factors have not yet been identified with any of the known lipogenic (i.e. stimulating fat formation and deposition) factors such as cholesterol and biotin. The first of these is a factor occurring in raw dried liver which has been reported by Blatherwick *et al* (47a, 48) to induce a fatty liver high in cholesterol when fed to rats. Extraction of the liver with water or alcohol prevented the fatty livers from developing. McHenry and Gavin (14) confirmed this work by feeding an alcohol extract of liver to rats on a low-fat, high vitamin diet. The possibility that the extract, a source of unisolated vitamins, stimulates the conversion of carbohydrate to fat has been suggested. This type of fatty liver has been shown to respond to choline plus inositol (10, 14) or to lipocaic (49). Handler (10a) has pointed out that choline alone is sufficient to obtain normal lipid concentrations even in the presence of liver extract or biotin plus folic acid, if the experiment was continued for 24 days instead of just a week. The similarity of the liver fraction fatty liver to the "biotin" fatty liver was suggested by McFarland and McHenry (49), early in their studies, but in more recent work these investigators reported marked dissimilarity between the two types.

In 1940, Ralli and Rubin (50) reported the induction of fatty livers in depancreatized dogs as a result of feeding an alcohol extract of raw meat. This suggested that in addition to a *deficiency* of pancreatic tissue or juice, or of lipocaic or choline, a *positive* dietary factor exists which influences the formation of fatty livers in these animals.

The mechanisms involved in lipotropic activity as well as the role of lipotropic factors in human nutrition will be discussed in the following issue of BORDEN'S REVIEW OF NUTRITION RESEARCH. The possible influence of lipotropic substances on human liver cirrhosis and the rationale behind modern diet therapy in liver disease will be presented.

REFERENCES CITED

1. ALLAN, F. N., D. J. Bowie, J. J. Macleod and W. Robinson. Behavior of depancreatized dogs kept alive with insulin. *Brit. J. Expt. Path. 5,* 75 (1924).

2. FISHER, N. F. Attempts to maintain the life of totally pancreatectomized dogs indefinitely by insulin. *Am. J. Physiol. 67,* 634 (1924).

3. HERSHEY, J. M. Substitution of lecithin for raw pancreas in the diet of the depancreatized dog. *Am. J. Physiol. 93,* 657P (1930).

4. HERSHEY, J. M. and S. Soskin. Substitution of "lecithin" for raw pancreas in the diet of the depancreatized dog. *Am. J. Physiol. 98,* 74 (1931).

5. BEST, C. H., J. M. Hershey and M. E. Huntsman. The effect of lecithine on fat deposition in the liver of the normal rat. *J. Physiol. 75,* 56 (1932).

6. BEST, C. H. and M. E. Huntsman. The effects of the components of lecithine upon deposition of fat in the liver. *J. Physiol. 75,* 405 (1932).

7. BEST, C. H., J. M. Hershey and M. E. Huntsman. The control of the deposition of liver fat. *Am. J. Physiol. 101,* 7p (1932).

8. BEST, C. H., G. C. Ferguson and J. M. Hershey. Choline and liver fat in diabetic dogs. *J. Physiol. 79,* 94 (1933).

9. BEST, C. H., M. E. Huntsman and J. H. Ridout. The "lipotropic" effect of protein. *Nature 135,* 821 (1935).

9a. BEST, C. H. and J. H. Ridout. The effects of cholesterol and choline on liver fat. *J. Physiol. 86,* 343 (1936).

9b. RIDOUT, J. H., C. C. Lucas, J. M. Patterson and C. H. Best. Lipotropic factors and the fatty liver produced by feeding cholesterol. *Biochem. J. 40,* 494 (1946).

10. BEST, C. H., C. C. Lucas, J. M. Patterson and J. H. Ridout. The lipotropic properties of inositol. *Science 103,* 12 (1946).

10a. HANDLER, P. Dietary factors in the regulation of liver lipid concentration. *J. Biol. Chem. 162,* 77 (1946).

11. DRAGSTEDT, L. R., J. Van Prohaska and H. P. Harms. Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. *Am. J. Physiol. 117,* 175 (1936).

12. ENTENMAN, C., and I. L. Chaikoff. Is choline the factor in the pancreas that prevents fatty livers in depancreatized dogs maintained with insulin? *J. Biol. Chem. 138,* 477 (1941).

13. ENTENMAN, C., I. L. Chaikoff and M. L. Montgomery. The preparation of fractions from pancreas that prevent fatty livers in depancreatized dogs. *J. Biol. Chem. 155,* 573 (1944).

13a. MCHENRY, E. W. and G. Gavin. The effect of biotin upon the synthesis of lipids in rats. *J. Biol. Chem. 140,* lxxxvii (1941).

14. GAVIN, G. and E. W. McHenry. Inositol: A lipotropic factor. *J. Biol. Chem. 139,* 485 (1941).

15. BEST, C. H. and J. H. Ridout. The effects of cholesterol and choline on deposition of liver fat. *J. Physiol. 78,* 415 (1933).

16. BEST, C. H., H. J. Channon and J. H. Ridout. Choline and the dietary production of fatty livers. *J. Physiol. 81,* 409 (1934).

17. MCHENRY, E. W. Vitamin B_1 and fatty livers. *J. Physiol. 89,* 287 (1937).

18. LONGENECKER, H. E., G. Gavin and E. W. McHenry. Fatty acids synthesized by the action of thiamine. *J. Biol. Chem. 134,* 693 (1940).

18a. BOXER, GEORGE E. and De W. Stetten, Jr. The role of thiamine in the synthesis of fatty acids from carbohydrate precursor. *J. Biol. Chem. 153,* 607 (1944).

19. BEST, C. H. and M. E. Huntsman. The effect of choline on the liver fat of rats in various states of nutrition. *J. Physiol. 83,* 255 (1935).

20. CHANNON, H. J. and H. Wilkinson. Protein and the dietary production of fatty livers. *Biochem. J. 29,* 350 (1935).

21. BEST, C. H., R. Grant and J. H. Ridout. The "lipotropic" effect of dietary protein. *J. Physiol. 86,* 337 (1936).

22. BEESTON, A. W., H. J. Channon and H. Wilkinson. The influence of the caseinogen content of diets on the nature of the "cholesterol" fatty liver. *Biochem. J. 29,* 2659 (1935).

23. BEST, C. H. and J. H. Ridout. Dietary casein and cholesteryl esters in liver. *J. Physiol. 87,* 55P (1936).

24. BEESTON, A. W. and H. J. Channon. Cystine and the dietary production of fatty livers. *Biochem. J. 30,* 280 (1936).

25. TUCKER, H. F. and H. C. Eckstein. The effect of supplementary methionine and cystine on the production of fatty livers by diet. *J. Biol. Chem. 121,* 4/9 (1937).

26. TREADWELL, C. R., M. Groothuis and H. C. Eckstein. The influence of supplementary casein, cystine and methionine on liver lipid content. *J. Biol. Chem. 142,* 653 (1942).

27. GRIFFITH, W. H. and D. J. Mulford. Choline metabolism. VII. Some dietary factors affecting the incidence and severity of hemorrhagic degeneration in young rats. *J. Nutr. 21,* 633 (1940).

28. MULFORD, D. J. and W. H. Griffith. Choline metabolism. VIII. The relation of cystine and of methionine to the requirement of choline in young rats. *J. Nutr. 23,* 91 (1942).

29. CHANNON, H. J., J. V. Loach, P. A. Loizides, M. C. Manifold and G. Soliman. The effect of proteins in the prevention of dietary fatty livers. *Biochem. J. 32,* 976 (1938).

30. TUCKER, H. F. and H. C. Eckstein. The effect of supplementary lysine, methionine and cystine on the production of fatty livers by high fat diets containing gliadin. *J. Biol. Chem. 126,* 117 (1938).

31. DUVIGNEAUD, V., H. Dyer and M. Kies. Relationship between the nature of the vitamin B complex supplement and the ability of homocystine to replace methionine in the diet. *J. Biol. Chem. 130,* 325 (1939).

32. DUVIGNEAUD, V., J. P. Chandler, A. W. Moyer and D. M. Keppel. The effect of choline on the ability of homocystine to replace methionine in the diet. *J. Biol. Chem. 131,* 57 (1939).

33. DUVIGNEAUD, V., J. P. Chandler, M. Cohn and C. Brown. The transfer of the methyl group from methionine to choline and creatine. *J. Biol. Chem. 134,* 787 (1940).

34. DUVIGNEAUD, V., M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds. The utilization of the methyl group of methionine in the biological synthesis of choline and creatine. *J. Biol. Chem. 140,* 635 (1941).

35. CHANDLER, J. P. and V. DuVigneaud. The comparative action of choline and betaine in effecting the replacement of methionine by homocystine in the diet. *J. Biol. Chem. 135,* 223 (1940).

36. STETTEN, DEW., JR. Biological relationships of choline, ethanolamine, and related compounds. *J. Biol. Chem. 138,* 437 (1941).

37. VAN PROHASKA, J., L. R. Dragstedt and H. P. Harms. The relation of pancreatic juice to the fatty infiltration and degeneration of the liver in the depancreatized dog. *Am. J. Physiol. 117,* 166 (1936).

38. DRAGSTEDT, L. R., J. Van Prohaska and H. R. Harms. Observations on a substance in pancreas (a fat metabolizing hormone) which permits survival and prevents liver changes in depancreatized dogs. *Am. J. Physiol. 117,* 175 (1936).

39. RALLI, E. P., S. H. Rubin and C. H. Present. The liver lipids and fecal excretion of fat and nitrogen in dogs with ligated pancreatic ducts. *Am. J. Physiol. 122,* 43 (1938).

40. MONTGOMERY, M. L., C. Entenman and I. L. Chaikoff. The liver lipids of dogs subjected to ligation of the external pancreatic ducts. *J. Biol. Chem. 128,* 387 (1939).

41. MONTGOMERY, M. L., C. Entenman, I. L. Chaikoff and C. Nelson. The role of the external secretion of the pancreas in lipid metabolism. *J. Biol. Chem. 137,* 693 (1941).

42. MCHENRY, E. W. and J. M. Patterson. Lipotropic factors. *Physiol. Rev. 24,* 128 (1944).

43. LUCAS, C. C. and C. H. Best. Choline—chemistry and significance as a dietary factor. *Vitamins and Hormones 1,* 1 (1943).

44. KAPLAN, A. and I. Chaikoff. Effect of autoclaved pancreas upon lipids of blood and liver in depancreatized dogs maintained with insulin. *Proc. Soc. Exp. Biol. Med. 34,* 606 (1936).

45. KAPLAN, A. and I. Chaikoff. The effect of raw and autoclaved pancreas on the liver lipids of the completely depancreatized dog maintained with insulin. *J. Biol. Chem. 119,* 435 (1937).

46. KAPLAN, A. and I. Chaikoff. The effect of choline on the lipid metabolism of blood and liver in the completely depancreatized dog maintained with insulin. *J. Biol. Chem. 120,* 647 (1937).

47. MONTGOMERY, M. L., C. Entenman and I. L. Chaikoff. Pancreatic juice is a rich source of the anti-fatty liver factor. *Am. J. Physiol. 148,* 239 (1947).

47a. BLATHERWICK, N. R., E. M. Medlar, P. J. Bradshaw, A. L. Post and S. D. Sawyer. Occurrence of fatty livers in rats fed a diet containing dried whole liver. *Proc. Soc. Exp. Biol. Med. 28,* 345 (1931).

48. BLATHERWICK, N. R., E. M. Medlar, P. J. Bradshaw, A. L. Post and S. D. Sawyer. Occurrence of fatty livers in rats fed a diet containing dried whole liver. The dietary production of fatty livers in rats. *J. Biol. Chem. 103,* 93 (1933).

49. MCFARLAND, M. L. and E. W. McHenry. The dietary production of fatty livers resistant to the action of choline. *J. Biol. Chem. 159,* 605 (1945).

50. RALLI, E. P. and S. H. Rubin. Effect of meat extract on fatty infiltration of liver in depancreatized and duct-ligated dogs. *Proc. Soc. Exp. Biol. Med. 43,* 601 (1940).

Editor's Note: In the light of more recent knowledge on the potency of natural and synthetic tocopherols (P. L. Harris, J. L. Jensen, M. Joffe, and K. E. Mason—Biological activity or natural and synthetic tocopherols, J. Biol. Chem. *156,* 491 [1944]), it has been demonstrated that natural alpha tocopherol is approximately 50 per cent more potent than synthetic *dl*-alpha tocopherol. This represents the current status of the subject discussed in BORDEN'S REVIEW OF NUTRITION RESEARCH, March, 1947—page 9 under the heading Vitamin E: Sources.